Submitted in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy, in the Faculty of Physiology, Columbia University.

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IS THERE A GASTRO-INTESTINAL MOTOR HORMONE?

Michael George Mulinos, A.B., A.M., M.D.

New York, N. Y.

April 15, 1929

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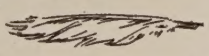

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TO MY ALMA MATER

This Is Respectfully Dedicated

IS THERE A GASTRO-INTESTINAL MOTOR HORMONE?

The facts brought forward in the following pages seem to suggest that one day this question will be answered in the affirmative. So far, investigations have been carried on along two lines. The one concerns itself with the vagus substance liberated whenever the pneumogastric is stimulated; the other, with a substance elaborated at a distance from the gut, which, carried by the blood to the vagus endings in that viscus, produces vagus-like effects. The latter is a true hormone; the former, a new type of substance for which the name Parasympatheticokinon is suggested.

Many investigators have isolated a substance from the gastro-intestinal tracts of animals, which injected intravenously stimulates the gut. It has been suggested that this stimulant is identical with the substituted ammonium compound, cholin. The German investigators, in particular, have found that cholin injected intravenously, produces phenomena identical with those obtained by stimulation of the parasympathetic division of the autonomic nervous system. Because of the fact that this substance is found in the gut; because it is obtainable in amounts roughly proportional to the activity of that organ; and because intestinal segments washed free from

cholin, no longer react to atropine but regain that activity upon the addition of traces of cholin, it has been suggested that cholin is the hormone of gastro-intestinal motility.

Significant is the suggestion that stimulation of the vagus nerve does not affect the anatomical nerve endings directly, but acts upon the receptor substance, or myoneural junction, through the medium of a chemical substance liberated by the nervous impulse. This hypothesis finds its analogue in the observation of Loewi that stimulation of the vagus of an isolated heart imparts to the perfusate vagus-like properties. These he called "Vagusstoffe".

The second question under consideration concerns the existence of a substance answering more closely the classical description of a hormone. The answer to this question resolves itself into a consideration of the experiments of others not yet corroborated by personal work, and the experiments performed by me during the past four years. Professor Carlson and his co-workers have found that both hungering and diabetic dogs have hypermotile stomachs. When blood from these animals is transfused into normal dogs, the stomachs of the latter become hypermotile. Here is proof positive that the gastric hypermotility accompanying hunger and diabetes is due to a substance circulating in the blood.

My own experiments support this hypothesis, but only indirectly. I found that when an animal is rendered hypoglucemic by means of insulin, there occurs a marked hypermotility, and rise in tonus of

the empty stomach. This hyper-activity can be extinguished by the intravenous injection of glucose solution. I say "extinguished" because the activity of the stomach soon returns to a state presumably such as it would have enjoyed, had not insulin interrupted it. The re-establishment of a normal, or hyperglucemic level has stopped the production of a substance which has a stimulatory effect on the stomach. Furthermore, it was found that a diet deficient in some essential but as yet unidentified substance prevented the development of this insulin gastric hypermotility, despite the fact that with this diet the stomachs of the animals contracted as usual when empty. The hypothetical substance formed by insulin in animals receiving a normal diet, is apparently no longer elaborated.

The experiments described in the text were performed, for the most part, on normal, unanesthetized animals. We have endeavored to obtain proof that stimulation or depression of the stomach does or does not involve the elaboration of a hormonal or a vagal substance. It was argued that if a given procedure were to stimulate (or depress) the stomach, the circumstances connected with the stimulation (or depression) and their subsequent removal might lend support to the hypothesis herein advanced.

With this problem in mind, we looked about for procedures which are, by reputation, adequate gastro-intestinal stimulants or depressants. As a result, it was soon found that previous work in this field had to be repeated in detail because of lack of agree-

ment between the reported results of others, and our own. This fact has been especially annoying, because it was often found that a procedure which in other hands stimulated or depressed the stomach, failed to do so in ours.

There is, therefore, very little in the work previously reported which is of positive value in elucidating the question at hand. The work of the past four years has served the purpose of a house-cleaning. There follows an outline of the work done; of the observations made; of the conclusions drawn; of the work contemplated for the future; and a hypothesis for the justification of the latter.

I. CHOLIN AS A MOTOR HORMONE FOR THE GASTRO-INTESTINAL TRACT.

(a.) Cholin injected into the veins of intact unanesthetized dogs has no effect on the motility of the empty stomach.

(b.) Cholin depresses the gastric activity which follows the ingestion of small amounts of food.

II. THE EFFECT OF FOOD UPON THE EMPTY STOMACH AND ITS RELATION TO HUNGER

(a). Small amounts of food cause a depression of the three types of hunger contractions described by Carlson.

(b). The depression is followed by a period of

stimulation lasting from half to one hour, which is:

(1). not affected by more food,

(2). not affected by intravenous glucose solution,

(3.) abolished by atropine, and which

(4). ends in a state of gastric quiet unaffected by more food.

(c). This post-feeding activity resembles:

(1). the activity due to insulin hypoglucemia;

(2). type III hunger activity of Carlson; and

(3). the hypermotility following the intravenous injection of hypertonic sodium chloride and sodium sulphate solutions.

III. THE INFLUENCE OF EXPERIMENTAL HYPERGLUCEMIA ON THE GASTRIC HUNGER MECHANISM.

(a). The normal hunger activity of the stomach is uninfluenced by the intravenous injection of large amounts of glucose solution,

(b). The post-feeding activity described under II is also uninfluenced by such injec-

tions. The conclusion is therefore drawn that the blood sugar level is not one of the controlling factors of the gastric hunger mechanism.

IV. AN ATTEMPT TO USE CATS ANESTHETIZED WITH AMYTAL IN THE INVESTIGATION OF THE EFFECT OF EXPERIMENTAL CHANGES IN BLOOD SUGAR ON THE GASTRIC HUNGER MECHANISM.

(a). Cats anesthetized with amytal retain a blood sugar level very close to the "normal" level. These animals respond to sensory disturbance by hyperglucemia, and to insulin by a fall in blood sugar.

(b). The stomachs of cats under amytal narcosis are in a quiescent state, even after large doses of insulin. It is concluded that amytal is not a good anesthetic for the investigation of this problem.

V. THE INFLUENCE OF INSULIN HYPOGLUCEMIA ON THE MOTILITY OF THE STOMACH.

(a). Unanesthetized, hungry dogs show an inincreased motility of the stomach un-

der the influence of insulin hypoglucemia. This gastic activity is:

(1). unaffected by food;

(2). abolished by the intravenous injection of glucose solution, and

(3). reappears as soon as the blood sugar has fallen to a sufficiently low level.

(b). A number of animals failed to show these effects. No explanation of this discrepancy is at hand, for all evident possibilities were tested and discarded.

(c). A new set of dogs was operated upon and the experiments repeated. These animals show the typical insulin effect described under (a). One dog of the previous group (b), retested, gave a typical post-insulin curve. Two possibilities present themselves:

(1). a seasonal variation. The failure dogs were run from September to January, the later group, (c), from then on.

(2). A dietary deficiency. The successful experiments were run on dogs fed meat, bread and vegetables, the failures on bread

and meat only. This latter diet is deficient in Vitamine B, which is known to have a marked influence on carbohydrate metabolism.

(d). A fourth series of experiments with controlled diets is now in progress to decide this point, (2).

VI. THE INFLUENCE OF INTRAVENOUS HYPERTONIC SALT SOLUTIONS ON THE GASTRIC HUNGER MECHANISM

(a). Intravenous injections of 20 per cent sodium chloride solutions cause an increase in the motility and a rise of the tonus of the stomach.

VII. EXPERIMENTS TO BE PERFORMED IN THE FUTURE

(a). The effect of insulin, salts and food upon the stomach whose vagi have been previously sectioned.

(b). Transfusion of blood from these animals on isolated stomach and intestine strips and on the heart.

(d). Concentration and isolation of the substance or substances responsible for the effects observed.

Even a casual inspection of the tracings soon convinces one of the resemblance of the activities elicited under the influence of insulin hypoglucemia, food, and hypertonic salt solution. Whatever the genesis of the stimulating substance may be in each of the three cases, it must act on the myo-neural junction or a point central thereto. This statement is based on the fact that atropine, in doses insufficient to affect the muscle, always destroys the movements.

If we postulate that under the influence of these procedures, a substance is elaborated somewhere in the body with para-sympatheticomimetic powers, we are forced to assume that the manner of production of this substance varies in each of the three cases mentioned. For example, if, under the influence of insulin hypoglucemia, the liver is made to elaborate such a substance, then the injection of a sufficiently concentrated solution of glucose into the blood stream, stops the process. But the injection made during the height of the salt or food activities, is without any effect. It might be argued that glucose solution, by raising sufficiently the blood sugar level, "depresses" the insulin activity. Just this point however, offers the best proof of the hypothesis that under the influence of insulin a substance is elaborated which stimulates the stomach. *When a glucose solution is injected into a vein of an animal the stomach of which is in insulin hypermotility, the hypermotility is instantly extinguished. However, the stomach is not abnormally depressed by the glucose because its activity now is the same as though no insulin had*

been given. There is simple extinction of a hyperactivity evidently superimposed upon the normal hunger contractions.

Another point in favor of this concept is the observation not yet fully checked, that the insulin hypermotility is absent in animals on a diet deficient in some essential element, despite the fact that the stomach itself shows good hunger contractions, and the animals are subject to the usual hyperglucemic symptoms. It is important to bear in mind that, whatever may be the cause of the absence of gastric hypermotility due to insulin in dogs fed a deficient diet, there is no longer produced the hypothetical vagus stimulating substance.

These are the salient points in favor of a hypothesis which seemed to be necessary to explain many of the facts concerning gastic motility, and which justify further work along this line.

CHOLIN AS MOTOR MORMONE FOR THE GASTRO-INTESTINAL TRACT

Magnus (1905) showed that an extract can be obtained from the gut wall, which, when applied to the bath fluid of an isolated strip of intestine, acts to raise the tonus and increase the activity of that tissue. A short time previously, Enriquez and Hallion (1904), during the discussion of another paper, mentioned incidentally that they had isolated a substance from the duodenal mucous membrane of dogs, which, when injected into other animals, resulted in stimulation of the gastro-intestinal tract of the latter. This substance proved to be heat stable and soluble in alcohol. Weiland (1912), suspended the intestinal tracts of dogs, cats and rabbits in warm Tyrode solution for one or more hours, and found that there was imparted to the bath fluid, the power of quickening the motility of another piece of isolated intestine. The action of this *watery extract* involves either the pendular movements, or the tonus, or both. It resembles pilocarpine in this respect, and like the latter drug, its action is abolished by atropine. The active principle was found to be stable in antiseptics; stable to prolonged boiling; soluble in alcohol and ether, but only slightly soluble in acetone; and easily precipitated with phosphotungstic and phosphomolybdic acids. Its action on isolated

strips of intestine is through Auberbach's plexus because it is antagonized by atropine. Injected intravenously, it lowers the blood pressure of the cat and rabbit. Immediately after the injection there is strong epigastric peristalsis; daily injections lead to a diarrhea without fever or pathological findings. In cats, after a bismuth-potato meal, intravenous injection of the extract causes increased antral peristalsis, quicker duodenal filling, and a marked stimulation of the pendular movements; but no stimulation of the large gut. From these observations Weiland concludes that "...dass die Darmwand selbst eine Substanz enthält, welche den Auerbach'schen Plexus erregt, zeigt, das wir auch für die automatischen Bewegung des Darmes eine chemische Ursache anzunehmen haben".

Le Heux (1918) repeated Weiland's experiments, and not only did he confirm his observations, but succeeded in identifying the active principle as cholin, a trimethyl-oxyethyl ammonium hydrate. He states that: The marked increase in activity imparted to the active fraction by acetylation, in conjunction with the formation of precipitates with phosphotungstic acid, and alcoholic solutions of mercuric and platinic chlorides, seems good evidence that the active principle is cholin, or else some analogous substance. The physiological effects of the purified extract, injected intramuscularly, compare very well with those obtained by the injection of pure cholin solution. Acetylation of each causes approximately the same increase in activity, about 100,000 times, upon the isolated heart.

Stimulated by these findings, le Heux (1920) takes to task the variable effect of atropine on isolated intestinal strips. He states that Magnus, Unger and Kress believe moderate doses of atropine paralyze the gut, while small doses initiate and regulate the pendular movements; that P. Trendelenburg obtained stoppage of the intestine of intact animals; that Hirz found the drug stops the gut of the rabbit, but acts irregularly on that of the cat; and that de Jude determined that the action of atropine does not depend upon the pH or the salt content of the bath fluid, and Liljestrand concluded that the action of the drug depends upon the state of the gut at the time of application.

Does the inhibition of the gut by non-paralyzing doses of atropine depend upon the presence of cholin in the gut wall? If so, then the removal of the cholin by perfusion of the intestinal strip over a sufficiently long time should render atropine ineffective in modifying the movements of the strip. In such preparations the contractions are small, and tonus waves are lacking. Does cholin, through its vagus stimulating action, bring about the variable and anomalous results seen in non-washed intestinal segments? The researches of le Heux seem to justify just such a hypothesis. Atropine, le Heux argues, is antagonistic to cholin. Could it not be that the cholin content of the strip being examined, has some influence upon the action of the added atropine? A non-paralyzing dose of the latter drug will depress or not, depending upon the amount of cholin present. If the strip were washed for several hours to remove the cholin, then

the action of atropine would manifest itself without antagonism; and if its depressing effects depend upon that antagonism, then atropine should be—(a) without effect on the washed strips, and (b) it should regain its effectiveness upon the re-addition of cholin. This is precisely what does occur.

According to le Heux, then, there is in the intestinal wall, during life, sufficient cholin to increase the activity of Auerbach's plexus. If this cholin is diminished in amount by oft repeated washings, atropine will cease to act in doses previously adequate, until a concentration is reached when the atropine paralyzes the nerves and smooth muscle directly. In the intact animal where there is no cholin depletion, depression is obtained with small doses of atropine. Thus does he explain the variable results obtained by different investigators.

Do these facts permit the classification of cholin as a hormone of gastro-intestinal motility? Abderhalden and Paffrath (1925) have confirmed le Heux' findings of free cholin in the gut wall, and support his contention that cholin be considered a hormone. They injected 150cc. of warm Ringer's solution intraperitoneally in a rabbit, and drained it off an hour later, recovering 75 per cent of it. This solution, assayed biologically, was found lacking in cholin. The same gut was removed from the body and one half of it was suspended in warm Ringer's solution to increase its activity; the other half was suspended in the same way at room temperature. The outer solution was changed every few hours and its cholin content deter-

mined. Both specimens gave the same total amount of cholin after 12 hours,—a maximum. The active segment however, yielded 90 per cent of the cholin during the first 6 hours in the bath; the quiescent segment gave 90 per cent of its cholin from the third to the ninth hours. Their general conclusion is that the amount of cholin obtainable at any one time is proportional to the activity of the gut, and that these results offer new support to le Heux' hypothesis.

Abderhalden and Paffrath's experiments do show one thing: that cholin is possibly a by-product of intestinal activity. He offers no explanation for his inability to obtain cholin from the intact animal by intra-peritoneal perfusion.

Le Heux (1921) reasoned that if cholin is to be considered a gastro-intestinal hormone it ought to have some influence on the gastro-intestinal tract of the intact animal when administered intravenously. He fed a barium meal to cats, and after determining the average emptying time of the intestine and stomach of the animals, he injected 10 mg. of cholin per kilogram of body weight intravenously in a 1 per cent solution and determined its effect on the emptying time. He reports immediate antral peristalsis following the injection; an increased rate of filling and peristalsis of the duodenum; and obviously increased gastric movements after one hour. The stomach emptied 15 minutes more quickly than in the control, and the barium reached the proximal colon from one-half to three-quarters of an hour sooner!

Kühlewein (1921) reported a marked improve-

ment to follow the intravenous administration of 5 to 15 mg. of cholin chloride per kilogram of body weight in cats whose gastro-intestinal tracts had been previously paralyzed by deep chloroform anesthesia lasting two hours. Arai (1922a) observed marked improvement in cats suffering from artificially induced iodine peritonitis or from gastro-intestinal paralysis due to laparotomy and purposeful handling of the gut, following the administration of cholin.

Zunz and Gyorgy (1914), and Arai (1922b) have shown that chloroform or ether anesthesia, or morphine, do not change the cholin content of the alimentary tract and conclude that the paralysis incident to these forms of anesthesia cannot be due to loss of cholin. Klee and Grossmann (1925) discussing the clinical efficacy of cholin, conclude that, to be active on the gastro-intestinal tract, it must be administered intravenously and fairly rapidly, because it is inert when given by mouth, sub-cutaneously or intramuscularly. They could find no evidence of its efficacy in gastro-intestinal disorders.

Spatz and Weichmann (1924) used cholin hydrochloride (Kahlbaum) in 600 mg. doses dissolved in 240cc. of saline, and found that the action of the intravenously administered solution upon the gastro-intestinal tract, as shown by the X-rays was inconstant. Most injections had no appreciable effect, though they were given routinely to post-anesthesia patients.

These attempts to apply in the clinic the findings of the laboratory, point suggestively to the fact that

intravenously injected cholin solutions have little or no effect upon the tonus and motility of the human gastro-intestinal tract. In considering cholin as a gastro-intestinal motor hormone, the question arises as to whether proof of its efficacy in motivating the gastro-intestinal tract is essential for such a classification. Reid Hunt (1915) demonstrated that intravenously administered cholin solutions are only transitorily effective in lowering blood pressure, and that only traces of the added cholin could be found in the blood ten minutes after the injection.

In view of the above reports, it was decided to test the effect of cholin hydrochloride solutions on the gastric hunger contractions and digestive activity of the dog's stomach according to the modified balloon method of Boldyreff (1904) described by Carlson (1912). Details of the method and the results obtained may be found in a paper by the writer, Mulinos (1926). The conclusions arrived at through those experiments are as follows:

(a) Cholin hydrochloride in 1 per cent solution injected intravenously or subcutaneously into dogs, has no demonstrable effect upon the gastric hunger activity with the method and dosage used.

(b) When the cholin is injected intravenously into previously fed dogs, the increased motility and high tonus incident to the feeding, are immediately depressed. When injected simultaneously with or just before or after the feeding, there is a marked delay both in the onset of the gastric activity and rise in gastric tonus. (For a discussion of the properties

and significance of the post-feeding activity of the stomach above described, the reader is referred to a publication by the author Mulinos (1927a).

In considering cholin a hormone of gastro-intestinal motility, it is obvious that we are confronted with a problem in some ways parallel to that of epinephrine. Doses of the latter substance which are effective as circulatory and cardiac stimulants, depress the activity of the gastro-intestinal tract. The adrenal glands therefore cannot be said to have any influence upon the normal circulation through the elaboration of a hormone. Nevertheless no one will challenge the claim of epinephrine to the title of hormone. The analogy between the two substances goes no further, however. We have seen that even large doses of cholin rapidly injected into the blood stream have little or no influence on gastro-intestinal motility, though they do have a profound and often dangerous influence on the circulation. Secondly, this substance, though quickly destroyed or eliminated by the organism, is very stable to procedures usually very detrimental to a hormone. Then again, cholin is produced within the organ upon which it acts or is to act. It has no known remote action such as every other hormone has, and after which property the latter derives its name.

The experiments of Loewi (1921) on the heart have, I believe, a direct bearing on this question. This investigator found that a contracting heart yields to its bath solution a substance which has the power to inhibit the contractility of another heart to which it

is applied, in a manner resembling vagus stimulation, and that the amount of this substance is increased by stimulation of the vagus. In describing his experiments, he carefully avoids calling the substance a hormone, but contents himself with a descriptive title: "Vagusstoffe". This vagus substance has long since been identified with acetyl-cholin, the acetic acid ester of cholin. So far, there have been performed many experiments attempting to establish the relationship between the vagus substance and acetyl-cholin, and the results are overwhelmingly in favor of an identity. Loewi and Navratil (1926) found that the vagus substance and acetyl-cholin are both destroyed by a saline extract of frog heart in a few hours, whence it is concluded that the destruction is due to an enzyme action and that therefore the vagus substance is an ester. On the other hand Lambert, Hennequin and Menklen (1927) using the modified Loewi technic of Kahn, found that inotropic effects were obtained more frequently; and appeared independently of the behavior of the donor heart under vagus stimulation, or may even be the reverse of its action. This same perfusate from a heart the vagus of which had been strongly stimulated, had no chronotropic effect.

Acetyl-cholin possesses two properties which render the hypothesis of its role as vagus substance very attractive indeed. One is its great instability in alkaline solutions, and more especially in the blood; and the other is that, biologically, it is the most active substance known. The activity of the parasympathetico-mimetic cholin is increased approximately 100,000 times by acetylation, and this reaction is the one most

often used for the determination of cholin in biologic fluids; (le Heux (1918), Hunt (1915), and others). Acetyl-cholin has a demonstrably depressing effect upon the perfused frog's heart in a concentration of 1×10^{-9}, Dale (1914). When it is injected intravenously in mammals in increasing concentrations, there is at first a fall in blood pressure without cardiac slowing, then a more marked circulatory depression and cardiac depression. These effects are evanescent, lasting usually from one-half to one minute depending on the strength of the dose and the rapidity of the injection. The drug is so rapidly hydrolysed that a large amount can be administered by vein without any appreciable effect on blood pressure until the rate of injection is considerably increased.

Zuntz and la Barre (1927) found that "vagal" Locke's perfusate when mixed with normal oxalated blood of a cat, and then injected into a cat with reduced circulation, caused a slowing of the heart rate. If contact between the "vagal" Locke's solution and blood lasts more than two minutes, the inhibitory effect on the heart rate is diminished or lost. The arterioles are 100 times more sensitive to acetyl-cholin than the heart, 4×10^{-6} mgm. producing a fall in blood pressure; Clarke and White (1927).

The question of interest to us now is, of course, whether there are any serious objections to considering acetyl-cholin as the "vagus substance" of the gut. Granit and Bonsdorff (1927) found that a rabbit's heart with the vagi intact, when perfused with Ringer's solution and the perfusate collected before and

during a period of vagal stimulation, produced inhibition of the frog's heart six, of eight times. This effect was abolished if the frog's heart had been previously treated with atropine. These same investigators (1927b) curarized a rabbit under ether anesthesia; then denervated the stomach by cutting the vagus as it entered the abdomen, and by section of the splanchnics. A balloon inserted into the stomach failed to show that vagus stimulation in the neck caused a substance to be carried from the heart to the stomach causing vagus effects. The following experiments throw some light on the question.

These experiments were performed on cats under amytal anesthesia, Mulinos (1928); and it was found that the solution which contained 0.0001 per cent acetyl-cholin in 0.9 per cent sodium chloride when allowed to flow into a femoral vein at the rate of about 0.5cc. per minute had little or no effect on the blood pressure. The blood pressure fell definitely but without cardiac slowing when the rate of injection reached 0.8 to 0.9 cc. per minute. These were adult males weighing between 2 and 3 kilograms, so no relationship could be established in regard to weight. In this connection it must not be assumed that the lack of cardiac slowing is of any significance, for it has been found since these experiments were performed that amytal is not without effect on the cardiac vagus mechanism. The amount of the drug injected had no influence on the motility of the stomach. Dale (1914) has determined that the vasodilation from cholin and its esters is produced in organs supplied by the nervi

irrigentes and errigentes, and also in the rest of the arterial system including the limbs.

After the administration of atropine, cholin and its derivatives no longer cause a fall in blood pressure. If the dose administered is sufficiently high, acetyl-cholin after atropine causes a rise in blood pressure. This rise cannot be repeated by further injections of the drug and does not occur if nicotine had been previously administered. Still larger doses paralyze the voluntary muscle myo-neural plates. These vagus-, nicotine-, and curare-like actions are common to all ammonium derivatives, and suggest a possible chemical relationship between these structures. In the words of Professor Dale (1914), page 188:

"The question of a possible physiological significance in the resemblance between the action of cholin esters and the effects of certain divisions of the autonomic nervous system, is one of greatest interest, but one for the discussion of which little evidence is available. Acetyl-cholin is, of all the substances examined, the one whose action is most suggestive in this direction. The fact that its action surpasses even that of adrenin, both in intensity and evanescence, when considered in conjunction with the fact that each of these two bases reproduces those effects of involuntary nerves which are absent from the action of the other, so that the two actions are in many directions at once complementary and antagonistic, gives plenty of scope for speculation. On the other hand there is no known depot of cholin derivatives corresponding to the adrenin depot in the adrenal medulla, nor indeed, any evidence that a substance resembling acetyl-cholin

exists in the body at all .... The possibility may indeed be admitted, of acetyl-cholin or some similarly active and unstable ester, arising in the body, and being so rapidly hydrolysed by the tissues that its detection is impossible by known methods...."

From the above considerations it becomes obvious that the vagus substance of Loewi may be acetyl-cholin and that this same substance may be the one liberated whenever the gastro-intestinal vagus is stimulated. The fact that intravenously injected acetyl-cholin is without effect on the motility of the gut would seem to disprove this contention. However, there are a number of considerations which nullify the above criticism. In the first place the vagus substance is produced in the gut wall itself and is not carried to it by the blood from some remote depot. Secondly, the vascular effects of the liberated substance may not become manifest due to the rapid destruction of the acetyl-cholin by passage through an organ such as the liver.

To test out this hypothesis, a solution of acetyl-cholin was injected into a branch of the portal vein or into a branch of the superior mesenteric artery, of amytalized cats, the same as in the experiments cited above. It was found that when the acetyl-cholin solution was allowed to flow into the arterial branch leading to the gut, a balloon previously inserted into the intestinal loop supplied by the artery now gave evidence of intestinal stimulation. There was no fall in blood pressure despite the injection of relatively large amounts of acetyl-cholin directly into the splanchnic

area. To our surprise injection of the solution into a branch of the portal vein in amounts which would have a profound effect on blood pressure when injected into a systemic vein now had no effect whatever until the rate of injection was increased. A half cc. of the solution per minute into the femoral vein gave an obvious fall in blood pressure. Eight cc. of this same solution were injected into the portal circulation at the rate of 1 cc. per minute without the slightest effect on blood pressure. It was only when the solution was allowed to flow in at approximately 1.5—2.0 cc. per minute that the systemic pressure fell. It is thus seen that the blood pressure reducing properties of acetyl-cholin do not exclude its possible production in the gut wall. It is hoped that the experiments to be discussed will throw some light on whether the vagus substance is produced as a result of vagus stimulation as it is in the heart, and is therefore not a hormone, or whether it is the cause of what we know as the effects of vagus stimulation.

In recapitulation, it may be said that it is not improbable that vagus stimulation yields acetyl-cholin or some analogous substance which in turn brings about vagus effects by stimulation of the myo-meural junctions of the tissue in which it is produced. It seems quite evident that if acetyl-cholin or other substance is so produced, it cannot be considered as a hormone. It must be considered as a new type of substance,—as the connecting link between the nerve end and the myo-neural junction—present in the tissues in some inert, complex, but easily decomposed pre-

cursor. Its function as an activator of the parasympathetic nervous system suggests the name of *Parasympathcticokinon*. The general class of such substances enlarged to include the sympathetic division of the autonomic nervous system and the peripheral extension of the cerebro-spinal axis, might be called *Neukokinons*.

SUMMARY

(1). Cholin, and acetyl-cholin, given intravenously do not stimulate the stomach.

(2). Cholin intravenously depresses the increased contractions of the stomach incident to food.

(3). Acetyl-cholin can be given in greater concentration and at a faster rate, into the portal circulation, than in a peripheral vein, without a fall in blood pressure.

(4). The blood pressure reducing powers of cholin and acetyl-cholin do not preclude their production in the gut by vagus stimulation.

THE INFLUENCE OF EXPERIMENTAL CHANGES IN BLOOD SUGAR ON THE GASTRIC HUNGER CONTRACTIONS

Introduction:

In the course of experiments on the gastric motility of dogs in insulin hypoglucemic shock, my attention was arrested by the behavior of the stomach under these circumstances. The phenomena observed were correlated and an interpretation attempted. More and more was I forced to the conclusion that these gastric contractions must be due to some stimulating substance elaborated under the disturbing influence of insulin. Since glucose injections do not affect the normal gastric activity at all; and since the hypoglucemic hypermotility is extinguished by such injections, and in its place appear normal hunger contractions; it is evident that some gastric stimulating substance has ceased to be produced. The following report details the experimental procedures and results.

THE INFLUENCE OF HYPERGLUCEMIA ON GASTRIC MOTILITY.

Bulatao and Carlson (1924) working by the balloon method of recording gastric contractions, injected concentrated solutions of glucose intravenously into dogs. They found that when 1 cc. of 50 percent glucose solution per kilogram of body weight was administered intravenously there was depression of gastric

tonus and cessation of motility. Cane sugar administered in like amounts had no such effect. They discuss the significance of these observations and conclude that satiation incident to food may be due to gastric quiescence caused by an alimentary hypoglucemia. This hypothesis is supported by their observation that during insuling hypoglucemia there is increased gastric tonus and motility, and by the fact that starving animals show a high degree of gastric tonus, and an activity, much greater than that usually observed in the animal under consideration. Luckhardt and Carlson (1914) showed that whereas blood from a normal dog had little or no influence on the the behavior of the stomach of another dog when given intravenously, the blood from dogs which had been starved for various periods increased the motility and raised the tonus of the recipient's stomach.

EXPERIMENTAL:

This report is based upon over 50 experiments on 9 female dogs. The animals were operated on under morphine-ether narcosis and aseptic precautions. A fistula of the stomach was made, choosing a relatively avascular part of the gastric fundus. This was drawn to the outside through a small incision in the left upper quadrant of the abdomen just beneath the ribs. During convalescence the animals were trained to lie on the experimental table with a balloon inserted into the stomach through the fistula opening. After a time the animals would lie quietly for hours while gastric contractions were recorded on smoked paper by means of a manometer attached to the balloon in

the stomach by a rubber tube. Owing to the strong contractions of the stomachs of these animals it was found necessary to substitute chloroform for the water in the manometer. During the experimental period the animals were kept in cages, and only occasionally were they allowed on the roof to exercise. They were fed on bread and meat with a generous supply of dog biscuits.

Experimental hyperglucemia was obtained by the intravenous injection of 50 per cent glucose solution. This was made up fresh every few days from Merck's glucose and distilled water. The injections were made into one of the leg veins by means of a syringe. Blood for the quantitive estimation of sugar was drawn from a vein in another extremity to obviate the possible contamination of the blood by sugar laden subcutaneous fluid. During the venapunctures there was no need to hold or otherwise restrain the animals as once they were trained they offered no objection to these or other more painful procedures.

No results obtained were recorded until all the experimental procedures could be performed without any demonstrable effect on the gastric contractions due to these procedures. The blood suger determinations were made according to the method of Shaeffer and Hartman (1920).

RESULTS:

The animals weighed from 8 to 24 kilograms. Each animal was studied sufficiently long to eliminate all disturbances upon the gastric tracings incident to

the procedures. The experiments in this group may be divided into 3 sub-groups as follows :

(a). The blood sugar level during periods of gastric inactivity, and during gastric hunger contractions of varying duration and intensity.

(b). The effect of intravenously injected glucose solution upon the gastric hunger contractions.

(c). The effect of intravenously injected glucose solution upon the digesting activity of the stomach.

(a). It occurred to us that if intravenously injected glucose solution, by raising the blood sugar concentration causes an inhibition of the gastric contractions, that there might be some variation in the blood sugar concentration of normal dogs to account for the varying activity of the stomach during any given period. The animals were studied for from 1 to 4 hours at a time, and blood for blood sugar determinations was drawn from a leg vein either every half hour routinely, or during periods of prolonged quiescence or activity. The protocols detailed below show that there are no variations in blood sugar to account for the variations in gastric activity recorded.

Dog, I, female, weight 25 kils.; health excellent.

	Date	Blood sugar p. c.	Tracing type
(1).	3-29-26	0.095	tetanus
		0.072	quiescent
		0.074	quiescent
(2).	3-30-26	0.070	strong type II
		0.063	quiescent
		0.068	quiescent

(b). In this series of experiments the blood sugar level was artificially raised by means of 50 per cent glucose solution injected into a vein. The amounts given varied from 1 to 2 cc. per kilogram of body weight. The smallest volume injected into an animal was 10 cc. and the largest 30 cc. The injections were made rapidly, none occupying more than three minutes, in order to raise the blood sugar to as high a level as possible. We were surprised to find that few objective signs of any disturbance were observed either during, or immediately following, the injections.

The tracings obtained under these conditions may be divided into two groups:

(I). Those experiments in which intravenous injection of glucose gave immediate depression, and

(II). Those in which no change occurred.

The first set comprises about 5 out of 50 tracings. The periods of depression varied from 5 to 30 minutes. That these depressions are of no significance is shown by the observation that no later than 45 minutes after the injection, the contractions invariably return to their previous intensity. At this time the blood sugar is at a much higher level than before the injection. Furthermore the greatest depression occurred in those animals in which the more or less strongly hypertonic glucose solution was accidentally introduced into the subcutaneous tissue about the vein causing pain. I do not believe that most of these depressions can be wholly accounted for in this way.

Blood for sugar determination was drawn at varying intervals before and after the injection of glucose solution. There was no correlation evident between the blood sugar values and the type of reaction, the degree of depression, or the promptness with which the gastric hunger contractions returned. Blood sugar values obtained after the stomach had returned to its pre-injection state as evidenced by the tracing, were always very high. This is clearly brought out by the following protocol.

Same animal as in previous experiment.

Date	Blood Sugar p. c.	Type of Contraction	Comment
(1). 5-10-26	0.097	tetanus	before injection
	0.202	tetanus	blood drawn ½ hour after injection
(2). 5-12-26	0.101	tetanus	before injection
	0.179	depression	20 min. after injection

In the first experiment (1), the blood sugar was 0.202 per cent when the stomach had already returned to full activity. In experiment (2), on the same animal, where less sugar was injected originally, the blood sugar value was only 0.179 per cent as opposed to 0.202 per cent and the stomach was still depressed. Surely no correlation exists here between the blood sugar value and the gastric hunger activity, whatever may be the cause of the depression. Furthermore, this animal's stomach usually showed no depression at all after large doses of glucose solution intravenously.

(II). In the remainder of the tracings, about 45 in all, the intravenously injected glucose solution had no demonstrable effect on the gastric hunger contractions. This was true for all types of activity, and height of tone. That the blood sugar was often raised to extreme heights, without causing gastric depression, is convincingly brought out by the following typical protocols:

Dog II; female; weight 9 kils.; health good.

Date	Blood Sugar p. c.	Type Tracing	Comment
(1). 5-9-26	0.085	type I	before injection
	0.086	tetanus	before injection
	0.380	tetanus	10 min after 20 cc. glucose sol. 50 p.c. intravenously
(2). 7-23-26	0.077	type I	before injection
	0.212	type I	20 min. after 10 cc. glucose sol. 50 p.c. intravenously
	0.108	quiescent	I hour after injection

In almost every case, where the blood sugar determination was made within 20 minutes after the injection, the blood sugar value was above 0.200 per cent. These figures leave no doubt that when the blood sugar level is raised above normal by artificial means, the activity of the stomach remains unaffected. We believe we are justified in concluding that *in nor-*

mal animals the blood sugar level is not one of the controlling factors of the gastric hunger mechanism.

(c). In the third series of experiments these same animals were fed 50 grams of lean meat while on the table, and the gastric hunger contraction were being recorded. During the feeding the tube from the gastric balloon to the manometer was clamped in order that the tracing be not disturbed by the movements of the animal. The dogs, which were starved for from 18 to 36 hours prior to the experiment, would eat the meat with avidity. After several repetitions of this procedure, the animals would lie back on the table and doze off apparently undisturbed by the insufficiency of the meal.

In a communication already published (Mulinos 1927a) the author described an increase in gastric activity and tone following the ingestion of food. Immediately after the feeding of 50 grams of lean meat to a dog there is a cessation of the gastric hunger activity with a fall in tonus which is the more marked the greater the activity and tonus of the empty stomach had been. If the animal is fed during a quiescent period, there is no preliminary fall in tone, and there is often a slight rise, probably due to the bulk of the meat. The entrance of the food into the stomach undoubtedly relaxes the tonically contracted stomach, for were it not so the encroachment of the bolus upon the balloon would result in an increase of pressure. This suggests that the quiescent empty stomach is not tonically contracted.

The preliminary fall in tone is followed by a gradual rise to a high level, and by the appearance of

steep, frequent contractions of varying intensity. This tremendous increase in tonus, and in activity, continues for an interval of 30 to 70 minutes, then suddenly ceases. Washing out the stomach, or inducing vomiting, at this time, shows that practically none of the meat has passed out of the stomach or has been digested. This behavior of the stomach will be mentioned again in a later chapter.

More meat fed to the dogs during the height of the above described activity now has very little effect upon it. Food given at the end of this period, when the stomach is in a state of quiescence or shows small type I contractions, also fails to have any effect. The stomach is refractory.

Intravenous injections of 50 per cent glucose solution were given to the dogs either just before or just after the feeding, when the gastric activity and tone were at their height; or during the period of quiescence which follows. In this series of experiments the glucose injections caused no depression of the gastric activity. The results may be summarized as follows:

(i). Though the sugar solution was injected just before a feeding the high blood sugar value had no demonstrable effect upon the promptness of initiation of the digesting period, or on its intensity.

(ii). Given at the outset of the digesting phase, it had no effect.

(iii). Given at the height of digestive activity, it never caused any depression.

(iv). Given during the quiescent period which follows the digesting curve, it proved to be quite inert.

A number of injections were made during a state of gastric quiescence, in the hope that the single observation of Hughson and Scarff (1924)—that gastro-intestinal stimulation in a cat followed the intravenous injection of hypertonic glucose solution—, might be confirmed. Our failure to obtain any such stimulation, and the fact that they observed it only once in many experiments, suggests that hypertonic glucose solutions given intravenously have no stimulating effect on the quiescent stomach.

Discussion:

How are the above findings to be interpreted? Luckhardt and Carlson (1924) found that blood from a starving dog, (animals kept without food for from 7 to 16 days), when injected into a normal animal, caused a rise in tonus and an increase in activity of the stomach of the recipient. Blood from dogs rendered diabetic by previous operation on the pancreas, had the same effect. The activity of the stomachs of both the starving and diabetic animals was much greater than the pre-experimental normal of these same animals. Blood from starving animals is poor in reducing substances and this fact supports the thesis previously mentioned, that a low blood sugar concentration increases the activity of the stomach. No such thesis can be advanced when the results with diabetic blood are considered.

Bulatao and Carlson (1924) offer the following reasoning to correlate the facts:

(Page 113) "We all know that the raising of the blood sugar level of normal animals leads to an increase in the glycogen content of the tissues, and there is some evidence that the same thing takes place in the diabetic animal when insulin is administered. We also know that, when available, glycogen or glucose is the first source of energy in the tissue activity. According to Woodyatt, forced intravenous injection of glucose leads to increased sugar oxidation. We therefore advance as a working hypothesis that the influence on gastric motility of hypoglucemia and of intravenous administration of glucose in normal and diabetic dogs, (with and without insulin), is due to changes in the energy metabolism of the gastric motor tissues when the available glycogen is reduced or sugar cannot be oxidized. So far, the only known facts that do not clearly fit the hypothesis are the augmentation of gastric tonus and hunger contractions by the transfusion of blood from starving and diabetic dogs into normal dogs, as recorded by Luckhardt and Carlson. This would seem to point to the presence in starvation and in diabetic bloods of some substance that stimulates the gastric hunger mechanism. In this connection we refer to a series of studies from Professor Magnus laboratory, which attempts to demonstrate that cholin is a motor hormone of the gut, acting on Auerbach's plexus. It is possible that the gastric motor tissues when they exidize lipoids in conditions of reduced glycogen, liberate cholin, which in turn may stimulate the gastric motor mechanism... We are of course aware of the probable complexity of the tissue changes accompanying hypo- and hyper-glycemia; be-

sides the tissue glycogen, the initial acidosis, and the energy metabolism factors."

It seems quite clear from the above discussion that if the utilization of glucose by the tissues results in gastro-motor activity that the intravenous injection of glucose should increase, not decrease the motility of the stomach. As a matter of fact it does neither. A starving and diabetic state increases the gastric motility. There is no obvious connection, therefore, between glucose mobilization and gastric motility. Furthermore, if some substance is elaborated under the condition of the experiments, and then acts upon the gut directly or indirectly through the intermediation of cholin, that substance is to be considered the motor hormone to the gut, and not cholin.

Suffice it to say that the strongest point in favor of a humoral mechanism of gastric motility is the abolition of that activity, whatever its origin, by the administration of minimal doses of atropine. The possibilities are that the activator in question stimulates the cells of Auerbach's plexus, the nerve endings or the myo-neural junction. Cholin, of course, can very well be the substance, except that all facts so far point to its being the *result*, not the *cause*, of the vagus stimulation.

There is also some evidence that not all the vagus nerve fibers which supply the gut are interrupted in the cells of Auerbach's plexus. Cushny (1918) states without further reference, that atropine paralysis of the vagus inervation of the gut is not complete, and that now, stimulation of the vagus is

still effective. Mulinos (1927b) showed that nicotine fails to completely paralyze the gastric and intestinal vagus, so that after as much as 0.5 gram of nicotine per kilogram of cat, stimulation of the vagus in the neck elicits a stronger response than before the administration of the drug.

Protocol:

Cat. Balloon in mid-jejunum; water manometer record of intestinal movements. Right vagus stimulated electrically. The cat was first etherized and then the brain and spinal cord were pithed. Artificial respiration instituted.

Right vagus stimulated 30 sec.: Rise in tone and increase in activity lasting 1 min.

Nicotine 10 mg. per kilo. intravenously: Marked fall in tone and cessation of all activity. Graph now a straight line.

Vagus stimulation 30 sec.: No effect.

Vagus stimulation, more nicotine, again vagus stimulation, then more nicotine without effect.

Vagus stimulation—5th time no effect.

Nicotine 10 mg. per kilo. I. V.; total now 50 mg per kilo.: Stimulation with rise in tonus and increase in activity, which continues

Vagus stimulation now slightly but definitely effective. Each new injection of nicotine, and each vagus stimulation causes a progressively greater increase in intestinal response.

> After 80 mg. nicotine per kilo. vagus stimulation elicits a greater response than did the first vagus stimulation.

It follows, therefore, that the substance we are discussing does not act on the central nervous system, for then, impulses coming down over that part of the vagus which is not affected by atropine, would go through and be effective despite the atropine. This statement is based on the questionable and improbable assumption that these vagal non-atropine sensitive fibers have some form of function on intestinal motility.

The difficulty of working with intact non-anesthetized dogs suggested that perhaps an anesthetic could be found which had little or no effect on the blood sugar of the animal. Amytal seemed just such a drug, and consequently a series of experiments were performed to test its efficacy.

ISO - AMYL - ETHYL - BARBITURIC - ACID (AMYTAL) AS A LABORATORY ANESTHETIC FOR CATS

Page (1923) was the first to draw the conclusion that amytal anesthesia has no effect on the blood sugar level of dogs and rabbits.. The dogs received by mouth, 0.100 to 0.125 gram of amytal per kilogram of body weight. Induction of surgical anesthesia required 1 to 3 hours and the anesthesia lasted from 18 to 24 hours. Rabbits required 0.08 to 0.10 gram of amytal by mouth and from 0.06 to 0.08 gram subcutaneously per kilogram. Anesthesia was induced in 10 to 15 minutes, but lasted only 3 to 6 hours. There was no disturbing influence on the blood sugar level; there was a "normal" response to splanchnic stimulation; hemorrhage caused hyper-glucemia; and insulin retained its normal blood sugar reducing power.

Since the publication of Page's experiments there have appeared a number of reports concerning the alleged innocuousness of amytal as regards the carbohydrate metabolism. The results reported are far from being unanimously favorable to the original claims. However this may be, it was decided to test out the effect of artificial alteration of the blood sugar level on gastric motility of cats under deep amytal nar-

cosis. A detailed description of the manner of injection of the drug and the behavior of the animals during the induction of the anesthesia will be found in a recent publication by the author (Mulinos 1928).

Forty-six experiments were performed on 21 apparently healthy cats. Before being used they were kept by necessity in close confinement for from 1 to 4 days. The diet consisted of meat, (liver or beef heart), mixed with bread. The animals were fed once a day, but to insure an empty stomach on the day of experiment, they were not fed. Nine of the 31 cats were males and 12 were females; their weights ranged from 1.5 to 3.5 kilograms. One experiment was performed on each of 5 cats; 2 experiments on each of 8 cats; 3 experiments on each of 7 cats, and 4 on 1 cat. After anesthesia was complete, blood was drawn by cardiac puncture into oxalate containing tubes and its sugar content determined by the method of Schaeffer and Hartmann (1920).

Most of the animals in this series received the drug subcutaneously. Several were injected intraperitoneally; and although the results in these differed in no way from those of the subcutaneously injected animals, it was thought inadvisable to inject the drug so near the organs the functions of which were being studied. Subcutaneously, the average effective dose was 100 mg. per kilo. Intraperitoneally 80 mg. per kilo. sufficed.

The interval between injections and anesthesia was a variable one, both in the different animals and in the same animal on different days:

Cat IV, female

Date	Dose Amytal mg. per kg.	Weight kg.	Hours to Anesthetize
Aug. 11, 1927	100	2.6	2.3
Aug. 17, 1927	100	2.4	1.5
Aug. 29, 1927	100	2.4	2.0

Of 46 anesthetizations, 20 were completed within an hour, the remaining 26 requiring from 1 to 2.3 hours. In general, with a constant per kilogram dosage, the greater the weight of the animal, the slower the induction and the less the depth of the anesthesia. The animals were treated gently and never forcibly restrained. The injection was made rapidly with a sharp needle. The animal was then set upon the floor and allowed to wander about the room.

The blood sugar concentration determined just after anesthesia is complete agrees well with the "normal" figures of Scott (1914). Hourly estimations indicate that the blood sugar remains constant for several hours (four). The conclusion may be drawn that during the induction of the anesthesia, amytal causes little or no change in the concentration of the reducing substance of the blood:

Cat XIII. Female. Weight 2.6 kgm.

September 19, 1927, 9 a. m., 0.13 gram amytal per kgm. Subcutaneously

Blood drawn at:	11:20	12:20	1:25	2:20
Glucose concentration, %:	0.09	0.08	0.091	0.086

This typical protocol shows how well the blood sugar concentration is maintained. Since surgical anesthesia is not complete after the fourth or fifth hours following complete narcosis, experiments performed on cats must not be carried beyond this interval unless the procedures performed are not obviously disturbing to the animals.

Slight sensory disturbances, such as the passage of a rubber balloon into the stomach by way of the esophagus, frequently caused the cat to cough or retch, even at a time when the corneal and pupillary reflexes were absent. The effect of such a procedure on the blood sugar concentration is shown in the following table:

Cat II, male, weight 2.9 kgm.

September 12, 1927, 9:10 a. m., 0.10 gram amytal per kgm, subcutaneously.

Blood drawn at :	10:30	11:30	12:30	1:30
Glucose concentration, %:	0.08	0.11*	0.112	0.106

*At 10:20 balloon was passed into the stomach via esophagus as for gastrogram procedure. Some retching. Animal obviously disturbed. The stomach showed no activity and a low tone throughout.

The above experiments may explain the diverse results of various observers working with amytal. How much the animal is disturbed during the course of an experiment is quickly mirrored in the blood sugar concentration. It is this very sensitivity of the

carbohydrate mobilizing mechanisms of animals under amytal anesthesia which renders them so difficult to work with, and casts doubts upon the results obtained; and which at the same time allows the results of such experiments to be interpreted as if performed on "normal" unanesthetized animals. Most of us would not expect a disturbed animal's blood sugar concentration to remain unchanged.

As would be expected, insulin in doses of about 2 units per kilogram of body weight reduced the blood sugar concentration of amytalized cats:

Cat VII, female, weight 3.3 kgm.

October 7, 1927, 9:05 a. m., 0.1 gram amytal per kgm. subcutaneously

Blood drawn at:	10:20	11:25	12:30	1:25
Glucose concentration %:	0.086	0.059*	0.035!	0.05

* Insulin, a total of 5 units subcutaneously at 10:25.

! Despite such low blood sugar values, no amytalized animal ever had convulsions.

These experiments suggest that amytalized animals can respond to insulin in the same direction as do "normal" animals as regards their blood sugar concentration.

Five of the 21 animals died during the course of the anesthesia. All of these had received insulin subcutaneously. Two of the 5 received 0.1 gram of amytal per kilogram, and insulin. All five animals died from respiratory failure. That the insulin may play

some part in the death by respiratory paralysis is suggested by the following protocol:

Cat XIV, female, weight 1.7 kgm.

Hours after insulin:	0	1	2	3
September 19, 1927. Amytal 0.130 gram per kilo; insulin 3 units.				
Glucose conc. %	0.130	0.086	0.033	0.024*
October 12, 1927. Amytal 0.100 gram per kilo; insulin 2 units.				
Glucose conc. %	0.084	0.028	0.012*	dead

* No convulsive seizure.

Death from insulin hypoglucemia under amytal narcosis may be prevented, and the animal recover, upon the institution of artificial respiration. This was actually the case in 12 experiments not included in this report.

Balloon studies of the gastric activity of these animals agreed without exception in showing a quiescent state. Very seldom were any contractions evident, and when present, were so feeble and evanescent as to be worthless for the purposes of this investigation. In spite of the fact that amytalized cats respond "normally" to conditions affecting the mobilization of glucose in the blood, this problem cannot be elucidated on such animals.

Conclusion:

Amytal is not a good anesthetic for the study of gastric motility.

Amytal anesthesia does not raise the glucose concentration of the blood of cats either during the induction stage, or for four or five hours after anesthesia is complete. It does not prevent the appearance of hyperglucemia from sensory stimulation, nor does it interfere with the blood-sugar reducing powers of insulin.

THE EFFECT OF FOOD UPON THE EMPTY STOMACH AND ITS RELATION TO HUNGER

So far, all the conditions we have studied in their relationship to gastro-intestinal activity have been uniformly negative. Our only justification in studying them was the reports in the literature that these conditions stimulated or depressed gastric activity. There follows a discussion of a number of conditions which we have found to stimulate or depress gastric activity, and these will be studied in detail.

Following the procedure described earlier under cholin, we made a large number of observations on the behavior of the normal empty stomach following the ingestion of food. The observations were begun under the direction of Professor Carlson on four dogs; they were continued on nine dogs while in residence at the University of Minnesota; and confirmed on six dogs since coming to Columbia.

The animals were in good health during the period of observation. A gastric fistula was formed as already mentioned, and after the wound had healed and the animal trained to lie quietly, a number of observations were made to determine the "hunger activity" of the stomach. After insertion into the stomach, the

balloon was fixed in place by a broad tape passing about the animal. The tube bearing the balloon is thereby anchored. This procedure was deemed advisable in view of the possibility of having the balloon carried past the pylorus into the duodenum, for the contractions of this organ resemble somewhat those under consideration in this study. Introducing the balloon into the stomach by way of a fistula offers the least possible psychic disturbance to the animal; minimizes reflex changes from the mouth and the esophagus; and makes possible comparable daily readings and undisturbed oral feedings with the balloon in situ.

RESULTS:

After the animal had quieted and a period of gastric hunger contractions had been recorded, it was fed fifty grams of meat. Previous to each observation the animals were starved for from 24 to 48 hours. Consequently they ate the proffered meat and looked for more. At first it was thought a serious drawback that the animals should show so much disturbance of their equanimity because of insufficient food. It was found, however, that they soon became accustomed to the procedure and as soon as the meat had been eaten, would lie back and remain quiet. The excessive salivation (drooling and licking of chops) evident with the first few experiments, disappeared in the later ones. The writer is sure that the results reported here were not due to any psychic disturbance. For further details reference may be made to the published experiments (Mulinos 1928). In that report it was stated that no evidence of the establishment of a con-

ditioned reflex to this type of motility could be adduced, although the animals often showed evidence of expecting the food, when for some reason it was withheld. Since then, two animals have been studied in detail in regard to this phenomenon. "Brownie" was run and fed for 86 consecutive experiments. One minute before feeding 50 grams of meat, a ticking alarm clock was placed just behind the animal's head and left there for the remainder of the experiment. Although the stomach responded to each feeding as described below, the clock had no disturbing effect on this gastric response at any time. "Spotty" was run for 26 consecutive observations, with analogous results.

After a period of gastric hunger contractions was recorded, the tube to the manometer was clamped, the animal fed, and then the tube released. This procedure prevents marring of the tracing by the respiratory activity incident to feeding.

When about 50 grams of lean meat are fed to a dog whose gastric hunger contractions are being recorded, there follow three stages of activity, which have already been described in some detail on page 38.

We have already seen that these gastric contractions which follow the ingestion of small amounts of food are depressed by the intravenous injection of 10 mg. of cholin in 1 per cent solution, per kilogram of body weight; and are unaffected by a large rise in blood sugar caused by the intravenous injection of 1 cc. of 50 per cent glucose solution per kilo. Meat is not the only food which will elicit these contractions,

for it was found that bread moistened with water and fed in 50 gram amounts had an effect similar to that of the meat. Furthermore, meat suspended in water and squirted into the stomach through the fistula caused the same increase in activity as that fed by mouth. This suggests that this activity depends only upon the stomach directly and does not depend upon impulses to the stomach from the cerebrum or the taste-smell apparatus. That simple distension is insufficient to elicit the response is supported by the observation that water or 0.9 per cent sodium chloride solution injected through the fistula, have no power to reproduce the meat curve. Twenty cubic centimeters of 50 per cent glucose or of Karo syrup injected into the stomach during active hunger contractions depressed this activity without subsequently augmenting the tone or movements.

The period of increased activity, pages 38-39, following the intake of food, resembles closely the rise in tone and increase in rate of contraction which appear after the administration of insulin, to be discussed a little later. Type III hunger contractions, as described by Carlson, and a curve resulting from the intravenous injection of 1 cc. of 16 per cent solution of sodium chloride (isotonic with 50 per cent glucose) per kilogram of body weight also resemble this post feeding activity, and will be discussed in detail below.
Discussion:

When food is put into the empty stomach of a healthy adult dog under the conditions of this experiment, the activity of that organ is markedly altered.

The preliminary depression of the gastric hunger contractions is interpreted as reflex inhibition, analogous to that obtained by stimulation of the mouth or esophagus. The increase in tone and in activity following the preliminary depression has been hitherto looked upon as the digestive phase of the stomach. What is its cause? Simple distention of the balloon, or water or saline in bulk equal to the food eaten do not elicit this increase in activity. Both meat and water-soaked bread bring about this change, which comes on whether or no the dog is conscious of the fact that food has entered the stomach. Failure to "condition" the reaction suggests a purely peripheral origin of the mechanism responsible.

That this post-feeding activity is not a "digesting" activity, and that in all probability it is not an effort of the stomach to empty itself is supported by the following facts:

1. The increased activity begins immediately after feeding; it reaches its height just as chemical digestion is beginning, and comes to an end at the height of the chemical process, (about one hour later).

2. At the end of the period of increased activity, it is found that practically all of the ingested material is still in an easily recognizable, partly digested form.

Careful examination of a large number of tracings shows clearly that the activity here described to follow the ingestion of small amounts of food, and preceded by a short preliminary depression, is not a

continuation of the previous hunger activity, but a new type of activity. This is easily proven by the fact that the new curve is unlike the one it replaces in all cases except the type III hunger contractions of Carlson, and that it is just as easily elicited upon a quiescent organ. Furthermore, the gastric activity now under consideration is not a continuation of the previous "hunger" activity because now further feeding has no effect on the tone or activity of the stomach. The recently fed stomach may be considered, therefore, as a different organ with a different behavior, due to an altered physiological state. A mere description of the behavior of the stomach at any one time can tell us very little concerning its true physiological state. It is concluded, therefore, that *the response of an organ to a physiological stimulus is a better index of the physiological condition of that organ than is its mere behavior at any particular time.*

The tracings show that the first response of an empty, "hungry", stomach to food, is an inhibition of its activity and a fall in tonus. Were it to continue in such a state of inactivity and atonia, the individual might either feel satiated and discontinue eating too soon to meet his caloric needs, or the impulse to continue the intake of food would of necessity have to be continued by some non-gastric mechanism. The tracings make it obvious, however, that the stomach is not a passive bag into which food is thrown for no obvious reason. The preliminary fall in activity and in tone is soon followed by a period of increased tone and activity, coming on so soon after feeding that the con-

clusion seems justified that this does not represent an attempt on the part of the stomach to empty itself. It comes to an end at the height of gastric digestion, at a time when actual observation shows the food to be still in the stomach. It apparently does not belong to the so-called "digestive" phase of gastric physiology. If so, what is its function, and upon what conditions does it depend?

Whatever the genesis of the increased tone and activity of the stomach after feeding, the conclusion seems justified that this change has little or nothing in common with the ultimate processes of digestion and expulsion of the chyme from the stomach. "Hunger" says Carlson, "may be considered as the signal that the stomach is contracted for action. The unpleasantness of hunger leads to eating, eating starts gastric secretion, distends the organ, initiates the movements of gastric digestion and abolishes the sensation" (of hunger). It takes time to eat a full meal, one-half to one hour. If the above quoted statement were strictly true, the ingestion of a part of a meal would satisfy, and the sense of hunger be lost. This, however, is contrary to the common experience that the urge to ingest food increases with the first part of the meal. There is no obvious reason why the increased activity described above should not break through to consciousness and be felt as increased hunger.

Accordingly, the gastric hunger mechanism may be divided into two phases:

(a). The empty stomach phase, with its three

types of contractions described by Carlson, and which are destroyed by food.

(b) The predigestive phase which consists of increased gastric tone and activity, and is unaffected by the ingestion of food in moderate amounts. It runs its course in from one-half to one hour, varying in duration in different individuals, and in the same individual at different times. It is abolished by atropine.

The relationship between these contractions and the cholin content of the gastro-intestinal tract will be discussed later.

THE EFFECT OF HYPERTONIC SALT SOLUTION ON THE MOTILITY OF THE STOMACH

Hughson and Scarff (1924) made the observation that when a concentrated solution of sodium chloride was injected intravenously into cats, there was a great increase in the motility of the gastro-intestinal tract. With them, it was an incidental observation made during the study of the effects of the salt solution on the absorption of fluid from the gastro-intestinal tract. As far as we know, they have made no attempt to analyze the action further.

During our work with the effects of intravenously injected hyper-tonic glucose solutions, it was found that very occasionally there was a temporary depression of the gastric hunger contractions, which lasted for so short a time, and which depression disappeared long before the blood sugar had fallen to within normal limits, that it was thought the hypertonicity of the solution might be responsible for it. It was found, for example, that after the injection of the hypertonic solutions, the animals would get restless, often whine gently, or lick their chops. Within a minute or two they would quiet down again. Objectively, the amount of disturbance may be gauged from the following protocol:

Dog VII, female, weight 9.1 kgm. In fair health.

	Hemoglobin. per cent	Blood sugar per cent
At beginning of experiment	100	0.085
15 min. after 10 cc. 50 per cent glucose intravenously	93	0.276
55 min. after the glucose	100	0.156

To test out the possibility that the evanescent depression from glucose is due to the great hypertonicity of the solution, we decided to test out the effect of a solution of sodium chloride iso-tonic with 50 per cent glucose solution. Consequently the animals were injected intravenously with 1.0 cc. of 16 per cent salt solution per kilogram of body weight There followed no greater disturbance than after the glucose injections, despite the lower hemoglobin content of the blood:

Dog VII as above.	Hemoglobin per cent
At beginning of experiment	100*
15 min. after 10 cc. 16 per cent salt solution intravenously	88
60 min. after the injection	102

*The hemoglobin content of the blood was determined by comparing colorimetrically samples of blood diluted with 0.1 per cent HCl solution, considering the pre-injection sample as 100 per cent.

Following soon upon the injection of the hypertonic salt solution, the gastric activity and tone began to increase and so closely resembled the changes following feeding, that a hypothesis was at once formulated and tested. The presence of food in the stomach stimulates the gastric glands to secrete both NaCl, and from this hydrochloric acid. This of course means that there is an increase in the chloridion concentration in the wall of the stomach. If the increased motility of the stomach following the ingestion of food is due to the increase of chloridion in the stomach wall, then the intra-venous injection of strong NaCl solution should have the same effect. At this point, the mechanism of the former was believed to have been discovered. However, there are several serious objections to the acceptance of this explanation as the true one.

(a). Similar doses of the salt solution were injected into the vein of a dog with a Pavlov pouch of the stomach, but no evidence could be found that the procedure increased the fasting secretion, or that following the ingestion of food.

(b). The pre-digesting curve comes to an end at the height of chemical digestion, at a time when the stomach is secreting an optimum of chloridion.

(c). That the increase of chloridion concentration of the blood and consequently of the gastric glands is not the cause of the gastric activity in question is clearly shown by the fact that the same gastric activity is elicited by th intra-venous injection of 1 cc.

of 16 per cent sodium sulphate per kilogram of body weight. These "salt" tracings are unaffected by food; they persist after the hemoglobin and the chloridion content of the blood have returned to normal; they are obtainable in animals (cats) which have been decapitated and de-spinalized; and they are abolished by atropine. They shall be referred to later on in the general discussion of the possible causes of gastric stimulation.

ON THE EFFECT OF INSULIN HYPOGLUCEMIA ON THE ACTIVITY OF THE STOMACH

Earlier in the discussion, there was mentioned the fact that large variations in the activity of the normal empty stomach were not related to the blood sugar level of the blood. A little later we saw that raising the blood sugar level to great heights by the intravenous injection of 50 per cent glucose solution, had little or no effect on the intensity and course of the gastric hunger contractions. The increased tone and activity which follow the ingestion of small amounts of food, or the intravenous injection of 16 per cent salt solution, are also unaffected by a rise in the blood sugar concentration. Whatever the genesis of these contractions, is seems safe to assume that they are not dependent upon the blood sugar concentration, if the latter remains normal or rises. We shall see that the same holds true for a lowering of the sugar concentration of the blood, in spite of the fact that a new type of activity can be called forth by a sufficiently low blood sugar.

The experiments in this group may be conveniently divided into three groups. Group I was completed

at the University of Minnesota. Groups II and III were completed at Columbia.

Group I. The same animals were used for these experiments as for those concerning the influence of injected glucose. The animals were in good health, despite inadequate exercise. They were fed a mixture of bread and chopped beef, with a liberal allowance of dog biscuit. Before receiving insulin, the animals were usually starved for from 24 to 48 hours to insure an empty stomach, but more especially to diminish the available liver glycogen. In all other respects the experimental procedures were like those already described. In fact these experiments were interspersed with those already described.

About one-half to one hour after the administration of an adequate dose of insulin subcutaneously, usually one unit per kilogram of body weight was sufficient, the activity of the stomach increases, accompanied by a rise in tonus. This activity so resembles the pre-digestion tracing, and that following the intravenous injection of hypertonic salt solution, that it is indistinguishable from them. It always precedes convulsive movements, and as far as we could determine, it is always accompanied by weakness which varies from a slight drag of the hind legs to complete paresis.

This marked hypermotility of the stomach under the influence of insulin hypoglucemia is of interest from many points of view. Cannon, McIver & Bliss (1924) showed that cats under the influence of insulin, produced enough epinephrine secretion by

stimulation of the adrenals glands, to accelarate the denervated heart. This was demonstrated both on anesthetized and normal animals. Now it is fairly well established that epinephrine injected subcutaneously can increase the blood sugar concentration of normal or insulinized animals, and that it can so increase the blood sugar concentration of the latter as to enable an animal in hypoglucemic convulsions, to recover. It can do this in concentrations too low to affect the heart rate or the blood pressure of the animal. Furthermore, epinephrine can be injected into the vein of animals at a rate insufficient to affect the cardiovascular apparatus, and yet have a profound influence on the motility of the gut. It is therefore difficult to correlate Cannon's findings with ours, unless we postulate the production under insulin hypoglucemia, of a gastro-motor substance sufficiently powerful to activate the gut in the presence of the depressing influence of epinephrine. At any rate, the amount of epinephrine produced under these conditions is insufficient to depress the gut or to mobilize sufficient glycogen to prevent convulsions.

The insulin hypermotility resembles also type III hunger contractions as described by Carlson. The tracings are straightforward and easy to read. As soon as the blood sugar level rises above an apparently critical level, this activity is at once depressed, or more accurately, it is extinguished, for the depression may be temporary. There were six animals in this group, and all showed this effect when one or more units of insulin were injected subcutaneously.

The following protocol is illustrative of the whole group, and shows well that the degree of glucemia varies at the onset of the gastric activity.
Dog I, female, weight 24 kgm. Starved 24 hours.

Date	Blood sugar per cent	Gastric activity	Comment
4-3-26	0.092 0.034	type I tetanus	Before insulin 30 min. after 20 units insulin sc.
4-8-26	0.085 0.053	quiet tetanus	Before insulin 55 min. after 10 units of insulin sc.

These hypoglucemic contractions are extinguished by glucose, only to return again. That the rate of fall of the blood sugar concentration may play some part in the genesis of these contraction, is clearly shown by the following protocol:

Dog II, female, weight 12 kgm. Starved 34 hours.

Date	Blood sugar per cent	Gastric activity	Comment
4-3-26	0.095 0.043	type I tetanus	Before insulin 35 min. after 10 units insulin sub-cut.

		70 min. after insulin, and
....	quiet	15 cc. 50% glucose I. V.
0.130	tetanus	20 min. after glucose injection.

Gastric tetanus was very unusual for this animal except under the influence of insulin. The tetanus observed with a blood sugar concentration of 0.130 is true "insulin" tetanus, and not a return of type III hunger contractions.

Group II consists of 86 experiments performed on four healthy female dogs at the Medical Center. Conditions at first were the same as for group I, except that the animals were fed by a diener, supposedly a mixed diet, and in that they were allowed to run on the roof as often as weather permitted. The animals were very friendly, and soon learned to lie quietly for many hours without being disturbed.

To our surprise, these experiments proved to be absolutely negative. Forty to 70 minutes after the injection, in three of the four dogs, of insulin, the blood sugar concentration has fallen, and the animal is in hypoglucemic shock. There is no change in the gastrogram tracing, however, in any direction. In dog I, the gastric hunger contractions were so frequently type III, that it took a large number of experiments to prove to our satisfaction that insulin hypoglucemia had no effect on the motility of the stomach. This par-

ticular animal proved very refractory to insulin, as much as 30 units given sub-cutaneously having only a comparatively slight effect on blood sugar. Sixty units of insulin failed to cause any noticeable unsteadiness.

To explain the discrepancy between the results of group I and of group II, we thought it possible that some dietary factor had entered into it. Consequently, the animals were removed from the roof to the animal room on the departmental floor, and fed the same type of diet as the animals of Group I had received, namely, bread and chopped beef. Despite this more close duplication of conditions, the motility of the stomachs of these dogs remain unaffected by the insulin hypoglucemia. The normal hunger contractions of the stomach behaved as was to be expected. There were good spontaneous contractions of all types and if anything, we were left with the impression that these stomachs were hyperactive. The gastric hunger contractions were uninfluenced by the intravenous injection of glucose. The stomachs behaved to food as already described, although the reactions were not so marked as one would be led to expect from the intensity of the empty stomach contractions.

Hemoglobin determinations made on the blood drawn for glucose determinations show that as the blood sugar concentrations decreases, the hemoglobin concentration increases. This is in confirmation of the work of Drabkin and Edwards (1924). The following protocol illustrates both these points in detail. Hemoglobin estimations were made by the method

already described, and are represented by relative figures only.

Dog II, female, weight 8 kgm. In good health. Exceptionally lively.

	Date	*Blood sugar*	*Hemo-globin*	*Gastric activity*	*Comment*
1.	9-17-28	0.091		Type III	Before insulin
2.	"	0.073		Type I & II	One hour after 8 units insulin
3.	"	0.059		Weak Type III	1.5 hrs. after ins
4.	"	0.045		"	2 hrs. after ins
5.	"	0.050		"	2.5 hrs. after ins
6.	9-19-28	0.063		Quiescent	20 min. after 8 units insulin
7.	"	0.049		Quiescent tone higher	1.3 hr. after ins
8.	"	0.049		Quiescent tone low	1 hr. 50 min. after ins.
9.	"	0.050		Quiescent tone low	2 hr. 15 min. after ins.
10.	10-1-28	0.093	100	Quiescent	Before insulin
11.	"	0.049	107	"	1 hr. after 20 units insulin
12.	"	0.039	106	"	1.5 hrs. after ins
13.	"	0.036	109	"	2 hrs. after ins. mod. shock
14.	10-10-28	0.135	100	Type III tone cm.	Before insulin
15.	"	0.130	97	"	1/2 hr. after 20 units insulin
16.	"	0.099	109	" less intense	70 min. after insuln
17.	"	0.090	112	" tone 2 cm.	1 3/4 hrs. after insulin
18.	"	0.096	112	" "	2 1/4 hrs. after insulin

After we were satisfied that this group of 4 animals would not behave as did group I, as regards the motility of the stomach, we decided to repeat the ex-

periment on another group of dogs, keeping conditions as nearly the same as possible. Two dogs were prepared for the experiment by a preliminary gastrostomy.

Group III of this series was composed of the two new animals, and of dog I of group II. The latter had had a long rest of two months between experiments and was in excellent health. Preliminary runs showed that the first of the new animals developed no motility of the stomach which could be due to insulin hypoglucemia.

About this time we began adding canned vegetable soup to the diet. All three animals received food from the same mixture of bread, chopped beef and canned vegetable soup. The next few tracings of new dog I showed changes bearing undeniable signs of disturbance from insulin. Within a short time, and really as soon as the animals had completely recovered from the operation and had become thoroughly trained, the stomachs of these animals behaved as did those of group I, under the influence of insulin hypoglucemia. About one hour after the injection of the insulin, the gastric tonus would rise, and the stomach would become hypermotile, and would keep so until the animal either had convulsions, or was given glucose by vein or by mouth. New dog II proved to be so sensitive to insulin, that 5 units often sufficed to give it convulsions within one-half hour after its injection.

At first it was thought that we had discovered the cause of the previous failure—Lilly's insulin—

for now we were obtaining the gastric hypermotility with Mulford's insulin. A trial of Lilly's insulin on these animals soon convinced us that both the Lilly and the Mulford insulin act alike. Is the new diet the cause of the change?

Dog I of group II seemed a good experimental subject for the test, for she had not only been studied in great detail before, but she had been receiving this same diet for about three weeks before we were ready to use her for an experiment. Much to our surprise and delight, the stomach of this dog now showed the typical insulin hypoglucemic reaction described in group I. Just at present the number of confirming experiments is far from convincing. They are very suggestive, however, and point to the possibility that something lacking in the diet of the dogs of group II has been added to that of group III, and that that substance is contained in the canned vegetable soup.

There is good evidence that the flow of gastric juice in dogs is greatly diminished or entirely inhibited, and in pigeons the lessened amount of gastric juice is almost free from pepsin, when diets low in vitamine B are given, (Miyadere, 1921; Danysz-Michel and Koskowski, 1922). The inhibition of flow of gastric juice in dogs is possibly due to the lack not of vitamine, but of some stimulating substance which is absent from vitamine-free diets; thus the administration of alcohol produces an immediate flow of gastric juice.

It was first observed by Funk (1914 a, b), that polyneuritis in pigeons was the more rapidly produced the greater the amount of carbohydrate fed to the birds, whilst starved birds did not acquire the disease. A corresponding increase in protein did not produce the same effect. This suggests that the vitamine is specifically used in carbohydrate metabolism.

The experiments of Cowgill (1921) are of interest in this direction. He found that dogs fed a diet deficient in vitamine B soon lose their appetite almost completely, and this is at once restored by the administration of small amounts of yeast extract or other preparation containing vitamine B. Later this writer (1926) found that the gastric hunger activity of dogs suffering from vitamine B anorexia had disappeared, and concluded that this gastric atonia and inactivity was the cause of the anorexia. Erma Smith (1927) was unable to confirm these findings.

The suggestion is made at this time that a sufficient amount of vitamine B is necessary for the proper functioning of the motor mechanism of the stomach, under the unnatural conditions of insulin hypoglucemia.

DISCUSSION

In considering the elaboration of a neurokinon in the wall of the gut, in response to a nervous impulse, there arise two questions worthy of discussion. If the contractions of the gut are myogenic, and consequently independent of nervous elements, we are forced to assume that the stimulus to contract originates within the muscle cells themselves, or in the immediate non-neural environment, such as changes in temperature, pH, ionic distribution, drying, and so on. The contraction of a single cell must set up new stimuli of sufficient strength to stimulate the adjacent muscle cells in such orderly manner to yield a coordinated contraction. To do this, the contracting cell must either elaborate a substance which diffuses to and stimulates the adjacent cells, or the contracting impulse is conducted from cell to cell as in the heart, despite the absence of syncytium. There is no evidence that either of these suggestions has any basis, as indeed there is still much doubt that myogenic contractions occur at all.

On the other hand, if the impulse to contract originates in nerve tissue outside the muscle cell, we must account for several phenomena which the hypothesis does not readily explain.

(a). When a non-toxic amount of pilocarpine-HCl is added to the bath fluid in which is contracting an isolated strip of intestine, the tissue goes into spasm. This is promptly relieved by amounts of atropine which leave the muscle apparently unaffected, for now the strip of intestine is contracting rhythmically and as well as before any drug was added. The atropinized strip is now pharmacologically isolated from its motor nerve elements. Of course the objection may rightly be raised that atropine sensitivity is not a true definition of the physiological or anatomical vagus. The statement of Cushny (1918) that certain fibers of the vagus are not sensitive to atropine lends much weight to this objection, provided the statement can be put to successful experimental test.

(b). Nicotine solutions at first stimulate ganglion cells, then paralyze them. This does not hold for the cells in Auerbach's plexus if we assume that all the nerve fibers of the vagus are interrupted in this plexus, or that all ganglionic nerve cells are nicotine sensitive. In the case of the gut, nicotine in small doses renders the viscus insensitive to vagus stimulation. Larger doses gradually reverse the reaction, so that now vagus stimulation is even more effective than before the nicotine. No such conditions have been found to hold for the cardiac vagus, or for the sympathetic ganglia. Either the cells of Auerbach's plexus are insensitive to nicotine, and consequently the only known exception to its action, and an assumption difficult to admit; or there are fibers of the

vagus which go directly to the muscle cells of the gut, an assumption still more difficult to allow. There the question stands for the present.

As regards the origin of the heart beat, we can say with some assurance that it is both neurogenic and myogenic, or possibly and most probably, myo-neurogenic. The influence of the central nervous system on the heart beat is too well-known to require comment; but it cannot be denied that the heart can beat quite efficiently when completely isolated from the brain stem either anatomically by section of the vagus and the sympathetic, or pharmacologically by the administration of atropine and ergotoxine. The origin of the whole heart beat (auriculo-ventricular cycle) is in the sinus node, a structure more muscular than neural, and is thence conducted to the various chambers by a specialized muscle tissue, the bundle of His.

The analogy does not hold for the gastrointestinal tract only because of our meagre and inadequate knowledge of its intimate structure, the distribution of the intricate nerve plexuses within its walls, and the character of the co-ordinating mechanics of peristalsis. The anomalous behavior of the gastrointestinal tract to drugs suggests that we are dealing here with a mechanism peculiar to the gut, and that the chemistry of the structures under consideration is somewhat different from similar anatomic structures elsewhere.

Substances such as neurokinons and hormones which are elaborated in the gut wall and thrown into the blood stream either as secretions or by-products,

are separated from the general circulation by the liver. We have seen that the concentration of cholin necessary to lower blood pressure was greater when injected into the portal vein than into a systematic vein. Cholin could be produced either as a result of vagus activity or gut activity, or it may be the cause of what we know as parasympathetic activity. Its presence in portal blood need not manifest itself by a fall in blood pressure, thanks to the intervention of the liver. This is even more true in the case of acetyl-cholin, the substance at present thought to be most closely connected with vagus activity. Its tremendous activity, and its great instability, allow it at once to stimulate neighboring sensitive structures, and to have no effect on relatively distant but actually closely situated structures. Thus it is not surprising that stimulation of the cardiac vagus should have no effect on the isolated stomach by the liberated "Vagusstoffe," because the latter is both enormously diluted and rapidly destroyed by the blood. The fact that little or no vaso-dilation occurs when the gastro-intestinal vagus is stimulated is a point against the elaboration of acetyl-cholin in the gut wall, for the sensitivity of the capillaries to the drug is greater than that of the gut. However, a possible explanation is offered in that not all the capillaries are equally sensitive; and we have seen that those of the liver do not react at all to large doses of the substance. Furthermore, increased activity and vascularity go hand in hand.

If the hypothesis is correct, that what we term as vagus effects are due to some substance liberated

by the nervous impulse, then: (a) during vagus stimulation, the substance should be increased (Magnus and co-workers).

(b) atropine paralysis of the myo-neural junctions of the parasympathetic should render vagus stimulation ineffective, but the amount of substance released should not be changed.

(c) Pilocarpine stimulation of the gut should not materially increase the amount of substance formed.

(d) Portal blood allowed to flow into the vena cava during stimulation of the peripheral end of the abdominal vagus might show the effect of this substance on the heart.

The question of the hormonal activity of the gastro-intestinal tract is one which can be attacked much more directly because the "action at a distance" of the hormone raises the hope that one day it may be isolated. We can expect it to be of sufficient stability to be effective when the blood in which it is transferred to the gut from its depot, is perfused through an isolated sensitive organ. The evidence of the existence of such a hormone is meagre, but sufficient to stimulate persistent effort toward identification and isolation.

One must repeat the experiments in which the transfusion of blood from hungry and diabetic dogs into normal dogs yielded positive results, in order that the best evidence for a hormonal control of gastro-intestinal motility under these conditions may be corroborated. The experiments of Ivy on auto-

transplants of gastric pouches in dogs offer indisputable proof of the existence of a gastric secretory hormone. It may be histamine, secretin, gastric, or other substance. It is of importance here in this sense, that it is elaborated by the stomach in response to definite sensory impulses, and then circulates in the blood, returning to the stomach, after having passed through the liver. The hormone of gastro-intestinal motility postulated above may be produced in the stomach in response to definite stimuli such as food, and thence carried by the blood, in like manner, back to the stomach and the rest of the gut, especially to the large intestine. Stimulation of the large gut by distention of the stomach may be a hormonal phenomenon. Transfusion of blood from animals in insulin hypoglucemia, and animals after salt injections, whose stomach are hypermotile, must be made into normal dogs, to further test the validity of the hypothesis.

Assuming the existence of a gastro-intestimal motor hormone, we may ask upon what structures it acts to produce its effects. Does it depress an active gastro-intestinal sympathetic nervous system? This would be contrary to the present conception that that system is not in a state of tone. Does it act on the smooth muscle cells directly? The paralyzing effects of atropine preclude any such conclusion. It may act on the myo-neural junction directly, and be a parasympathetico-mimetic substance in the class of philocarpine, cholin, muscarine, physostigmine, etc.; or it may act on the neural constituents of the vagus

system, i. e. nerve ends, Auerbach cells, or the central nervous system. The latter can be easily eliminated. When the vagi are cut, the gastro-intestinal tract behaves as before except for a slight loss in tone, and the abolition of peripheral reflex effects. Furthermore, there is no gastro-intestinal vagus center in the medulla which might be specifically stimulated, without at the same time involving the cardiac vagus.

If our hormone acts on Auerbach's plexus, it probably does so through the intermediation of the hypothetical parasympatheticokinon. The latter should increase in amount. Were it possible to evulse the last cell station of the vagus, and then allow time for degeneration of the post-ganglionic fibers, solution of the problem at hand would be relatively simple.

EPILOGUE

The validity of a hypothesis depends upon its power to explain and correlate knowledge and stimulate the imagination. Our justification in elaborating the "neurokinon" hypothesis lies in its ability to explain in a more rational way certain physiological phenomena. The non-specificity of the nervous impulse becomes intelligible; the great activity of the latter in causing effects very much out of proportion to its strength receives a rational explanation; and the fatiguability of the myo-neural junction to prolonged stimulation may be explained on the assumption that the neurokinon precursor becomes exhausted.

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VITA

I was born in Cairo, Egypt, on November 24, 1897, of Greek parents. At the age of 2 years, I was moved to Greece, where I attended the Gymnasium, and learned to speak, and read, and write modern Greek. In 1907, when I was 9 years of age, my mother, two older brothers, and I came to New York to my father, whom I had seen once before (so they told me) but whom I did not remember.

Since then I have graduated from public school 87, Manhattan, De Witt Clinton High School, and Columbia College in 1921 with the Bachelor of Arts degree. I entered the College of Physicians and Surgeons in 1920, and in 1922 received the degree of M. A. in Physiology under Professor E. L. Scott. In 1924 I graduated in medicine and began a year of internship at St. Vincent Hospital, Erie, Penna. From July 1925 to December 1926 I was a Fellow, then instructor in Pediatrics at the University of Minnesota and the Mayo Foundation Graduate School of Medicine, and a candidate for the Ph. D. degree. From January 1927 to the present recording I have been an instructor in Pharmacology at Columbia under the direction of Professor C. C. Lieb. The following is

a list of my publications, all but the first of which are from Columbia:

Studies on Cholin as a Motor Hormone for the Alimentary Tract. Amer. Jour. Physiol. lxxvii, 158, 1926.

The Gastric Hunger Mechanism. Amer. Jour. Physiol. lxxxiii, 115, 1927.

Gastro-Intestinal Motor Response to Vagus Stimulation After Nicotine. Proc. Soc. Exp. Biol. and Med., 49, 1928.

Hemorrhage Hyperglucemia. Am. Jour. Physiol. lxxxvi, 70, 1928.

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